FIN Protocol

MACRO-INVERTEBRATES

Aim

To list and assess the relative abundance of macro-invertebrate taxa for a defined part of each ECN site.

Rationale

Macro-invertebrates are a very diverse group of organisms with a wide range of environmental tolerances and preferences and are generally abundant in freshwater habitats. Communities are therefore likely to show both qualitative and quantitative responses to a full spectrum of possible environmental changes. In most situations macro-invertebrates are also relatively easy to sample, qualitatively at least, and keys for the identification of most elements of the British fauna are readily available.

Such considerations have, for many years, led to the extensive use of macro-invertebrates in biological assessment of water quality. Water quality indices based on macro-invertebrates are generally derived from scores allocated to taxa according to their perceived tolerance, or intolerance of pollution (eg Woodiwiss 1964). The necessary level of identification varies between methods but the widely used BMWP score (Biological Monitoring Working Party 1981) requires only family level data.

RIVPACs (Wright *et al.* 1993) allows prediction of the fauna which would normally be expected to be present in a river site in the absence of pollution. This model thus allows a high degree of objectivity to be applied in the interpretation of biotic scores. Furthermore RIVPACs methodology may also be applied at any taxonomic level, from species upwards, and can be used to compute an index of environmental quality derived as a ratio of the observed community to that expected for relatively pristine conditions.

Methodologies for using macro-invertebrates to monitor water quality in rivers are thus well established but this is less true for still waters. Nevertheless certain groups, notably midges, have been shown to be very useful in systems of lake classification (Saether 1979) and subfossil remains of midge larvae have also been widely used in elucidating historical changes, both natural and anthropogenic, in lakes (eg Walker *et al.* 1991).

Abnormalities in the appearance of particular macro-invertebrate structures often result from environmental contamination by toxic chemicals. Most notably, high incidences of deformities in midges, mainly of their head capsules, have been linked to toxic pollution in both lakes and rivers (eg Warwick 1980). Archiving of material in ECN will allow such factors to be investigated in this and in other groups at some future time if a need becomes apparent and funds are available.

Method

Equipment

Most sampling will be carried out using a long-handled pond-net of the type commonly used in RIVPACs surveys (Wright *et al.* 1993). This should either conform with the most recent specification for RIVPACs (Environment Agency 1997) or with the standard FBA pattern with a square aluminium frame of 257 mm side and a mesh size of 1000 µm (see Appendix I, page 96). A bag-depth of 500 mm, which avoids 'wash-back', is recommended but the standard bag with an average depth of 254 mm is acceptable. Multifilament polyester nets are preferred to monofilament nets since they are softer and easier to empty, although they are more easily damaged and manufactured to less precise tolerances. Damaged nets must be repaired or replaced before use. Details of the net used at individual sites must be recorded and sent to the CCU.

Location

The area to be sampled should be representative of the section of river or littoral zone of the lake and should, wherever possible, coincide with the area used for other ECN measurements, (eg macrophytes). It should be an area where the major habitat types of the littoral zone of the lake, or reach of a river, can be sampled within the pre-determined sampling time (see under Sampling, below). Sampling areas which may be influenced by atypical local influences such as bridges, weirs, artificial banks or cattle-drinking areas should be avoided. The size of the sampling area will depend on the size and character of the water body and is therefore not prescribed.

The location of the sampling point must be recorded as a NGR to 10 m and marked on the sketch map.

Data on a range of physical characteristics of rivers will be important for the interpretation of data collected on invertebrates, especially in the context of RIVPACs. Some of these will be gathered as part of the ECN Aquatic Macrophyte Protocol (see page 66). However, measurements of the following physical characteristics should be made at the same time as the invertebrate sample is taken:

- water width (width of water surface (±0.5m) at right-angles to the channel);
- water depth (as the average of depths (±0.1m) measured at points one-quarter, half and three-quarters of the distance across the stream channel).

If the location of the invertebrate sampling point does not correspond with the macrophyte sampling location, then a separate measurement of the substratum characteristics (see Aquatic Macrophyte Protocol, page 66 and Chapter 3, pages 103–106) should also be made.

Sampling

Detailed prescriptions of sampling methodology cannot be provided because the equipment used and the tactics adopted will vary with the site characteristics.

Sampling tactics should include both kick-sampling of the streambed or the littoral zone of the lake, hand-searching, and sweep-sampling of any vegetation.

The standard total sampling time for a site will be 3 minutes, the standard time used in RIVPACs. The proportion of the total time which is allocated to each habitat type is proportional to the estimated surface area occupied by that habitat category. In addition there should be one minute of hand-searching for species such as river limpets. The same habitats should be sampled on successive occasions. Samples from different habitats are not kept separate.

Dredge sampling may be used as the primary sampling method in deep rivers or lake margins where it is impossible to sample adequately by other means. The Medium Naturalist's Dredge, described by Holme and McIntyre (1971) is recommended for use in RIVPACs and a similar type should be used for ECN. This comprises a rectangular metal frame, of aperture size 457 mm x 203 mm (dimensions need not be exact but the same pattern is to be used on each sampling occasion) to which a net bag is attached. The bag has a mesh of 1000 µm and a depth of 600 mm. It is protected by an open-ended outer skirt, which is constructed of more robust material. A tow-rope is secured by shackles to two lateral arms, also connected by shackles to the short sides of the rectangular frame. Details of its construction are given by Holme and McIntyre (1971).

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Five tows of the dredge are recommended. One throw should be almost parallel with the bank to collect marginal species. In rivers, the dredge should

be thrown downstream, since recovery against the current reduces lift during retrieval. Over coarse or compacted substrata retrieval should be in a series of short, sharp movements to cause maximum disturbance. Over finer or less compact surfaces a more rapid, even retrieval will cause the dredge to skim efficiently through the upper layers of the substratum. If the retrieval is too rapid however, the dredge will lift off the bottom and few animals will be caught. Wherever possible dredge sampling should be supplemented by pond-netting of any marginal habitats not sampled adequately using the dredge. The several dredge samples are not kept separate but if both dredge and pond-net sampling are used, samples collected by the different methods are kept separate because of the bulk of the material, mainly detritus, gathered by the dredge.

It is important that for each site the methodology is determined at the outset and is not varied subsequently, otherwise between-year comparisons will be impossible.

For both running and standing waters three samples each year are preferred, taken during the periods March–May, June–August, and September–November, since this procedure has been shown to give a reasonably comprehensive species list. However, if the minimum prescription for running waters of two samples per year is followed, these should be taken during the first, and then during one of the other two periods, with the choice of the second sampling being consistent between years. The minimum prescription for standing waters of a single sample per year requires the sample to be taken during the spring period, (ie in March–May).

Sample treatment

If samples are to be sorted live they **must** be kept cool in a refrigerator or cool box in the field and in a refrigerator in the laboratory. They **must** be sorted within 48 hours of collection.

Ideally, however, samples should be preserved in the field using 10% formalin (4% aqueous formaldehyde), preferably immediately after collection to prevent carnivores present in the sample from eating other specimens. The fixative hardens the cuticle of insects and oligochaetes and reduces the chances of disintegration during handling and storage. Fixative can be added either in the concentrated form, from small bottles stored in each sample container, or in a dilute form (10% formalin) from a suitable jerry can. In-house procedures for using formaldehyde are provided by the Environment Agency (1997). These procedures have been tested to meet statutory safety standards (COSHH); however, every laboratory using these procedures, or other procedures authorised by their own laboratory, must carry out its own safety assessment, tailored to its own particular conditions and facilities.

If concentrated fixative is used, put approximately 100 ml of undiluted formalin (40% aqueous formaldehyde) in a 150 ml screw-topped bottle in the laboratory, using a fume cupboard or fume extractor. Place a bottle of fixative in each sample container. In the field add sufficient fixative to the sample to result in a 10% formalin solution, taking into account the volume of the water sample. Replace the cap on the bottle containing the unused formalin and replace it in the sample container. This procedure must take place outside, in a well-ventilated area and **not** inside a vehicle. Adherence to this procedure ensures that the formalin is always double-sealed, prevents large volumes of fixative being carried in the same container and limits the total volume being carried to that required for sampling. Protective gloves must always be worn when handling concentrated formalin.

If dilute fixative from a jerry can is used, this is added to the sample in a well-ventilated area and **not** inside a vehicle.

The formalin is distributed through the sample by gently tumbling the container, having first made sure that the cap is secure. Some air must be left in the container to ensure that mixing is thorough and this must be done in a well-ventilated area or in a fume cupboard in the laboratory.

Samples must be left in fixative at least overnight to ensure that they have been thoroughly penetrated by the fixative. Subsequently samples may be left in formalin until they are to be sorted, or the fixative can be thoroughly washed out and the samples stored in 70% alcohol (industrial methylated spirit). Washing must be carried out in a fume cupboard or using a fume extractor.

Preservation in alcohol after fixation

If fixed samples are to be kept for more than a few months before sorting, they must be preserved by transferring them to alcohol. A 70% aqueous solution of Industrial Methylated Spirit (IMS) and 5% glycerol is needed for effective preservation. It is necessary to ensure that the residual alcohol concentration is adequate after allowing for the penetration of organic matter and dilution by displaced water; this is achieved either by changing the alcohol several times or by using alcohol with an initial concentration greater than 70%. The addition of sufficient 90% alcohol to provide an overlying volume roughly twice that of the sample will usually be adequate.

Sorting, identification and counting of specimens

Samples must be well-washed with water before sorting, using a fume cupboard or fume extractor for the purpose. All animals retained by a sieve of mesh size 500 µm are to be regarded as part of the sample and should be identified using the following criteria.

As far as possible all the invertebrates in the samples should be identified to **species** level; in cases where the necessary level of expertise is not available, **minimum** acceptable levels of identification are given in Appendix II (page 96). The revised checklist and coding system (Biological Dictionary Determinand Working Group 1989) should be used as the standard for ECN macro-invertebrates; this allows for identification at mixed taxonomic levels. All specimens should be archived after preservation in 70% alcohol in small vials; the addition of formaldehyde is not necessary at this stage. It is recommended that after sorting, all samples should be re-constituted, preserved, well labelled and archived.

Absolute numbers, or an estimate of absolute numbers (±10%) through sub-sampling, should be determined for each taxon identified. This will allow subtle changes in community structure to be detected. For the purposes of RIVPACs, an estimate of abundance at family level (with the exception of Nematoda, Oligochaeta and Hydracarina which should be reported in these 3 groups) should also be made using a log scale of abundance of 1–5 as follows:

1	1–9 animals
2	10–99 animals
3	100–999 animals
4	1000–9999 animals
5	>10000 animals

Author

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References

Biological Dictionary Determinand Working Group. 1989. *A revised coded checklist of freshwater animals occurring in the British Isles*, version 1. (Compiled by M Furse, I McDonald & R Abel). Unpublished software. London: Department of the Environment.

Biological Monitoring Working Party. 1981. *River Quality: the 1980 Survey and Future Outlook.* London: National Water Council.

Doughty, C.R. 1989. *A baseline study of acidified waters in Scotland.* Research Contract PECD7/10/104, Department of the Environment. Glasgow: Clyde River Purification Board.

Environment Agency. 1997. *Procedures for collecting and analysing macro-invertebrate samples.* Environmental Monitoring Programme: Biological Techniques, BT001. (Internal publication). Bristol: Environment Agency.

Holme, N.A. & McIntyre, A.D. 1971. *Methods for the study of marine benthos*. IBP Handbook No **16**. Oxford: Blackwell.

Saether, O.A. 1979. Chironomid communities as water quality indicators. *Holarctic Ecology,* **2**, 65–74.

Walker I.R., Smol, J.P., Engstrom, D.R. & Birks, H.J.B. 1991. An assessment of Chironomidae as quantitative indicators of past climatic change. *Canadian Journal of Fisheries & Aquatic Sciences*, **48**, 975–987.

Warwick, W.F. 1980. Palaeolimnology of the Bay of Quinte, Lake Ontario: 2000 years of cultural influence. *Canadian Bulletin of Fisheries & Aquatic Sciences*, **206**, 1–117.

Woodiwiss, F.S. 1964. The biological system of stream classification used by the Trent River Board. *Chemistry and Industry,* **11**, 433–437.

Wright, J.F., Armitage, P.D., Furse, M.T. & Moss, D. 1993 RIVPACs – a technique for evaluating the biological quality of rivers in the UK. *European Water Pollution Control*, **3**, 15–25.

Appendix I

Supplier

Details of suppliers of the standard FBA hand net (Model HN1) and other equipment are obtainable from the CCU.

Appendix II

Taxonomic identification levels required as a **minimum** for ECN. Table after Doughty (1989).

Phylum	Class	Sub-class	Order	Identification level
Platyhel-minthes	Turbellaria		Tricladida	Species except *Polycelis nigra/ tenuis* and *Dugesia lugubris/ polychroa*
Nematoda				Phylum
Annelida	Oligochaeta			Family
	Hirudinea			Species
Mollusca				Species except *Sphaeriidae* (genus)
Arthropoda	Crustacea	Malacostraca		Species
	Arachnida		Acarina	"Hydracarina"
	Insecta		Ephemeroptera	Species
			Plecoptera	Species
			Odonata	Species
			Hemiptera	Family
			Coleoptera	Family except Elminthidae (species)
			Megaloptera	Species
			Trichoptera	Species except *Glossosoma, Agapetus, Wormaldia* (genus) and Hydroptilidae (family)
			Diptera	Family except *Tipula, Eloeophila, Dicranota, Hexatoma, Pedicia, Atherix, Limnophora* (genus)

Specification of results and recording conventions

The measurement variables listed below are those required for each FIN sampling location at an ECN Site. Sites submitting data to the ECNCCU should refer to the accompanying Data Transfer documentation for the specification of ECN dataset formats, available on the restricted access Site Managers' extranet. Contact ecnccu@ceh.ac.uk if you need access to this documentation.

The first 4 key parameters uniquely identify a sample or recording occasion in space and time, and must be included within all datasets:

• Site Identification Code (e.g. R10)	Unique code for each ECN Site
• Core Measurement Code (e.g. FWC)	Unique code for each ECN 'core measurement'
• Location Code (e.g. 01)	Each ECN Site allocates its own code to replicate sampling locations for each core measurement (e.g. FWC 01, FWC 02 for different surface water collection points)
• Sampling Date (/time)	Date on which sample was collected or data recorded. This will include a time element where sampling is more frequent than daily

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Core measurement: Freshwater macro-invertebrates (FIN protocol)

The following variables are recorded from samples taken at a recommended frequency of three times per year for both rivers and lakes:

Variable	Units	Precision of recording
Site identification code		
Core measurement code		
Location code		
Sampling date		
Sampling start time	GMT 24-h clock	1 min
Sampling duration	mins	1 min
Sampling method	1-character code: P = Pond net D = Dredge	
Net mesh size	µm	1
Net bag depth	mm	1
Net mouth area	mm^2	1
Water width	m	0.1
Water depth (average)	m	0.1
For each species present:		
Species code	8-digit code(1)	(eg 04320704)
Species name	genus species	(eg *Mesostoma tetragonum*)
Numbers of individuals	count (2)	
For each family(3) present:		
Family code	8-digit code(1)	
Family name		
Abundance class	1-digit code (4)	

Notes

(1) ECN uses the 'revised Maitland' coding system (Furse *et al.* 1989) used by RIVPACs. A copy may be obtained through the CCU.

(2) This may be an estimate (±10%) by sub-sampling for large numbers of individuals.

(3) Abundance class should be determined at family level, except for Nematoda, Oligochaeta and Hydracarina for which abundance class should be recorded in these 3 groups.

(4) The species abundance coding system is as follows:

1 1 to 9 animals
2 10 to 99 animals
3 100 to 999 animals
4 1000 to 9999 animals
5 >10000 animals